Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 3223–3227, May 1986
Biochemistry

Cloning, sequence, and expression of bovine interleukin 2

(cDNA cloning/expression in *Escherichia coli*/protein homology)

DOUGLAS PAT CERRETTI*, KATE MCKEREGHAN*, ALF LARSEN*, MICHAEL A. CANTRELL*, DIRK ANDERSON*, STEVEN GILLIS†, DAVID COSMAN*, AND PAUL E. BAKER‡

Departments of *Molecular Biology and ‡Animal Health, †Immunex Corporation, 51 University Street, Seattle, WA 98101

Communicated by Lewis H. Sarett, January 9, 1986

ABSTRACT **Interleukin 2 (IL-2) cDNA clones have been isolated from both human and murine sources. We report here the isolation of a cDNA clone encoding bovine IL-2. This was accomplished by screening a cDNA library constructed from lectin-stimulated bovine lymph node cells, using a human IL-2 probe. Bovine IL-2 is composed of 155 amino acids and has a predicted molecular weight of 19,555. Alignment of the amino acid sequence with human IL-2 indicates that mature bovine IL-2 is composed of 135 amino acids and has a predicted molecular weight of 15,452. It has an amino acid homology of 65% with human IL-2 and 50% with murine IL-2. Bovine IL-2 is unique among IL-2 homologs in that it has a single N-linked glycosylation site. Biologically active bovine IL-2 was synthesized in an *Escherichia coli* expression system.**

Interleukin 2 (IL-2) is a lymphokine that induces proliferation of responsive T cells (1) and certain B cells (2, 3) via receptor-specific binding *in vitro*. Considerable data also suggest a similar function and mode of action of IL-2 on IL-2-responsive cells *in vivo* (4–7). By using IL-2-dependent murine T-cell clones, IL-2 from both humans and mice has been biochemically characterized. Both were found to be secreted as single polypeptides with molecular weights of 15,000–17,000 on $NaDodSO_4$/PAGE (8). Size and charge heterogeneity was found to be a function of variable degrees of glycosylation, which had no effect on activity (8). More recently, human and murine IL-2 have been cloned from cDNA libraries and functional lymphokine has been expressed in recombinant vector systems (9–14). The human cDNA encoded a polypeptide composed of 153 amino acids, the first 20 of which served as a signal sequence and were cleaved prior to secretion. The murine amino acid sequence showed approximately 60% homology to the human sequence.

Recent attempts to characterize IL-2 from other animal species, especially those of economic importance such as cattle, have been hampered by the fact that the activity of this lymphokine is partially species restricted. Since bovine IL-2 was found to have no effect on the murine T cells used for human and murine studies, bovine IL-2-responsive T-cell lines were generated and utilized to characterize the bovine lymphokine. As with the murine and human forms, IL-2 from cattle was found to display size and charge heterogeneity (15). This observation, the significant degree of homology between the human and murine amino acid sequences, and the fact that recombinant human IL-2 induces bovine T-cell proliferation, led us to examine the possibility of utilizing a human cDNA probe to isolate its homolog from a bovine cDNA library. Using this technique, we isolated a full-length copy of bovine IL-2 cDNA, capable of directing the synthesis of functional lymphokine in an *Escherichia coli* expression system.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Construction and Analysis of a cDNA Library. Polyadenylylated messenger RNA (mRNA) was isolated from 100 ml of bovine lymph node cells (10^7 per ml) stimulated for 17 hr with concanavalin A (Con A, 7.5 μg/ml). Procedures for RNA purification and cDNA library construction have been described (16, 17). Small-scale plasmid DNA preparations from pools representing approximately 2.5×10^3 transformants were digested with *Pst* I, electrophoresed on 0.8% agarose gels, blotted onto nitrocellulose filters, and hybridized with a ^{32}P-labeled human IL-2 cDNA probe corresponding to nucleotides 52–759 of the published sequence (9). Hybridizations were for 16 hr at 55°C in 6× NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M sodium citrate, pH 7) containing 0.1% Sarkosyl, 5× Denhardt's solution (1× = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin), 0.5% Nonidet P-40, 100 μg of denatured salmon sperm DNA per ml, and probe at 10^6 cpm/ml. Filters were washed extensively in 6× NaCl/Cit at room temperature and then washed for 1 hr at 42°C and for 1.5 hr at 55°C before autoradiography. Positive pools containing the largest hybridizing cDNAs were subdivided, and the process was repeated on pools of 500 transformants. Positive pools were then used in colony filter hybridization experiments to identify transformants that hybridized strongly with the probe.

DNA from the cDNA clones was subcloned in M13mp18 and mp19 bacteriophage (18) and sequenced by the chain-termination method (19, 20).

Expression and Analysis of Recombinant IL-2. An *E. coli* expression vector was constructed by inserting a blunt-ended (by T4 DNA polymerase) *Hgi*AI/*Pst* I fragment (encoding amino acids 22 to 135) from pBIL2-4 into pLNhumIL2 with synthetic oligonucleotides to provide an *E. coli* ribosomal binding site, a translational initiation site, and a codon for amino acid 21 (Fig. 2). The nucleotide sequences used for the ribosome binding site were based on sequences used to give high level expression of human IL-2 in *E. coli* (21, 22). The resulting recombinant plasmid, designated pLNBovIL2, was used to transform *E. coli* strain RRI (American Type Culture Collection no. 31343) containing plasmid pRK248cIts (American Type Culture Collection no. 33766), which has a gene encoding a thermolabile repressor of the λ phage P_L promoter. pLNhumIL2 was constructed by inserting a DNA fragment encoding human IL-2 into *Hpa* I-digested pPL-lambda (Pharmacia) with a synthetic oligonucleotide (M.A.C., unpublished results). pPL-lambda contains the λ P_L promoter on a pBR322-derived vector. During the construction of pLNBovIL2, DNA coding for human IL-2 was deleted. Expression of bovine IL-2 in *E. coli* and sample preparation for IL-2 assay were conducted as previously described (17) with the exception that cells were induced at an OD_{600} = 0.5

Abbreviations: IL-2, interleukin 2; bp, base pair(s).

by a shift to 42°C. Samples were lysed in $NaDodSO_4$ buffer and analyzed by gradient (10–20% acrylamide) $NaDodSO_4$/PAGE (23). Gels were stained with silver as previously described (24).

Bovine IL-2 Assay. BT2, a bovine IL-2-responsive T-cell line, was originally generated via an allogeneic mixed leukocyte reaction, and it was shown to display alloantigen-specific cytolysis several months after having been grown in bovine, simian, or recombinant human IL-2 (25). Samples were assayed for IL-2 activity by utilizing BT2 cells in a microIL-2 assay (26).

RNA Analysis. Polyadenylylated mRNA was isolated from Con A-stimulated and unstimulated lymph node cells as described (16). Samples were then electrophoresed in 1.1% agarose gels containing formaldehyde, transferred to nylon membranes (Hybond-N, Amersham), and hybridized with a ^{32}P-labeled RNA probe transcribed by SP6 RNA polymerase. We have found RNA probes to have greater sensitivity in blot hybridization analyses than nick-translated double-stranded DNA probes. The [^{32}P]RNA probe was synthesized from a 484-base-pair (bp) *Rsa* I/*Dra* I fragment (corresponding to nucleotides 22–506 and isolated from pBIL2-4), which was inserted into pGem-1 (Promega Biotec, Madison, WI). Hybridization and washing of blots were as described (16).

Genomic DNA Analysis. Genomic DNA was isolated from bovine peripheral blood leukocytes by standard techniques (27). DNA (10 μg) was digested with various restriction endonucleases and electrophoresed in 0.7% agarose gels, blotted, and hybridized at high stringency to a ^{32}P-labeled bovine IL-2 probe (27). The ^{32}P-labeled probe was made by nick-translating the 506-bp *Pst* I/*Dra* I fragment (corresponding to nucleotides 1–506, Fig. 1*B*) isolated from pBIL2-4.

RESULTS

Cloning and Sequence of Bovine IL-2 cDNA. Screening of a bovine cDNA library with a ^{32}P-labeled DNA fragment encoding human IL-2 resulted in several positive clones. One of these, pBIL2-4, was selected for further analysis. A partial restriction endonuclease map and a complete DNA sequence of the cDNA insert in pBIL2-4 are shown in Fig. 1. pBIL2-4 contains a cDNA insert of 775 bp, including a stretch of A residues corresponding to the poly(A) tail of mRNA. These A residues are preceded by the polyadenylylation/maturation signal, AATAAA (28). The sequence also has an open reading frame of 155 amino acids, starting with an initiator Met codon at nucleotide 18 and ending with a termination codon at nucleotide 485. This protein has a predicted molecular weight of 19,555. Comparison of the amino acid sequence with that of human IL-2 (12, 29) indicates that the amino-terminal residue of mature bovine IL-2 may be Ala-21. The mature protein would be composed of 135 amino acids and have a predicted molecular weight of 15,452. The 5′ end of the open reading frame encodes a region of 20 amino acids with many characteristics expected of a signal peptide for secreted proteins (30).

Expression of Recombinant Bovine IL-2. To determine whether the cDNA in pBIL2-4 encoded a protein with IL-2 activity, we constructed a plasmid, pLNBovIL2, designed to direct synthesis of mature bovine IL-2 under transcriptional control of the λ P_L promoter (Fig. 2). Cells, transformed with this plasmid or a control plasmid lacking the IL-2 insert, were grown at 30°C before heat induction of the P_L promoter by shifting the cultures to 42°C. The proteins synthesized by these cultures were analyzed by $NaDodSO_4$/PAGE (Fig. 3). A major new protein band (arrow), which is presumably

A

B

```
                                                            5'--CCTCAACTCCTGCCACA    17

Met Tyr Lys Ile Gln Leu Leu Ser Cys Ile Ala Leu Thr Leu Ala Leu Val Ala Asn Gly     20
ATG TAC AAG ATA CAA CTC TTG TCT TGC ATT GCA CTA ACT CTT GCA CTC GTT GCA AAC GGT     77
 *
Ala Pro Thr Ser Ser Ser Thr Gly Asn Thr Met Lys Glu Val Lys Ser Leu Leu Leu Asp     40
GCA CCT ACT TCA AGC TCT ACG GGG AAC ACA ATG AAA GAA GTG AAG TCA TTG CTG CTG GAT    137

Leu Gln Leu Leu Leu Glu Lys Val Lys Asn Pro Glu Asn Leu Lys Leu Ser Arg Met His     60
TTA CAG TTG CTT TTG GAG AAA GTT AAA AAT CCT GAG AAC CTC AAG CTC TCC AGG ATG CAT    197
                                     ▲
Thr Phe Asp Phe Tyr Val Pro Lys Val Asn Ala Thr Glu Leu Lys His Leu Lys Cys Leu     80
ACA TTT GAC TTT TAC GTG CCC AAG GTT AAC GCT ACA GAA TTG AAA CAT CTT AAG TGT TTA    257

Leu Glu Glu Leu Lys Leu Leu Glu Glu Val Leu Asn Leu Ala Pro Ser Lys Asn Leu Asn    100
CTA GAA GAA CTC AAA CTT CTA GAG GAA GTG CTA AAT TTA GCT CCA AGC AAA AAC CTG AAC    317

Pro Arg Glu Ile Lys Asp Ser Met Asp Asn Ile Lys Arg Ile Val Leu Glu Leu Gln Gly    120
CCC AGA GAG ATC AAG GAT TCA ATG GAC AAT ATC AAG AGA ATC GTT TTG GAA CTA CAG GGA    377

Ser Glu Thr Arg Phe Thr Cys Glu Tyr Asp Asp Ala Thr Val Asn Ala Val Glu Phe Leu    140
TCT GAA ACA AGA TTC ACA TGT GAA TAT GAT GAT GCA ACA GTA AAC GCT GTA GAA TTT CTG    437

Asn Lys Trp Ile Thr Phe Cys Gln Ser Ile Tyr Ser Thr Met Thr End                    155
AAC AAA TGG ATT ACC TTT TGT CAA AGC ATC TAC TCA ACA ATG ACT TGA TCACTAAGTGCCTCT    500

CATTTTAAACTATCAGGCTTTCTATTTATTTAAATATTTAAAATTTATATTTATTTTTTGATATATGTTTTCCTACCTT    579

TTGTAACTGTTAGTCTTAAGATGATAAATATGGATCTTTTAAGATTCTTTTTGTAAGCCCTACGGGCTTAAAAATTCAG    658

TTAAATTATTTATCCTGAAGTATTTATTTGTATATTGAATTTTTAAATATAATGTCTATGCAGGTCATTGACTAAAATT    737

ATTTAATAAAGTTGATGAATAAAAAACAAAAAAAAAAAA--3'                                         776
```

FIG. 1. Restriction map and nucleotide sequence of bovine IL-2 cDNA. (*A*) Partial restriction map of the cDNA insert in pBIL2-4. Coding sequences are boxed. The open box represents the proposed signal sequence, and the shaded box represents the coding region for mature protein. The *Pst* I sites in brackets were generated by the cloning procedure. (*B*) Nucleotide sequence and predicted amino acid sequence of bovine IL-2. The predicted amino terminus (Ala-21) of mature bovine IL-2 is marked with a star. The triangle represents a possible N-linked glycosylation site, and the 3′ polyadenylylation/maturation signal is underlined.

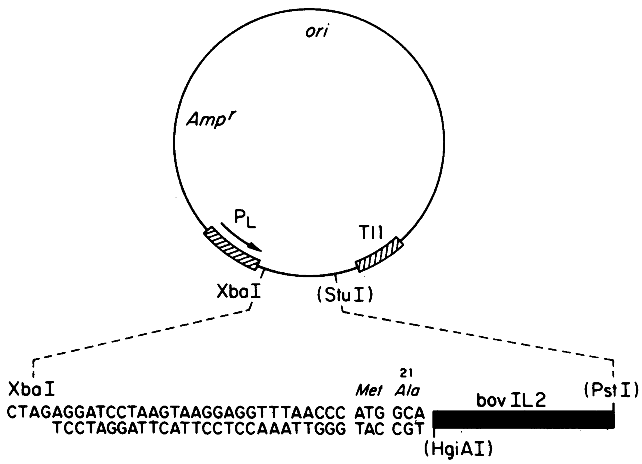

FIG. 2. Structure of the *E. coli* expression plasmid pLNBovIL2. The plasmid contains sequences derived from pBR322 containing the origin of replication (*ori*) and the ampicillin resistance gene (*Ampʳ*). The hatched boxes represent regions containing the λ P_L promoter used to direct transcription of IL-2 and the transcriptional terminator T11. The synthetic oligonucleotide containing a ribosome binding site, an initiator Met codon, and a codon for Ala-21 is shown fused to the coding region of bovine IL-2 (solid box).

bovine IL-2, continued to accumulate up to 20 hr. An apparent molecular weight of 15,250 for this protein is in good agreement with the molecular weight of 15,452 deduced from the DNA sequence. Densitometer scanning of silver-stained gels of total *E. coli* protein from an induced culture (Fig. 3, lane 4) indicates that bovine IL-2 represents approximately 10% of the cellular protein. Cultures that were induced for 20 hr (Fig. 3, lane 4) were simultaneously assayed for IL-2 activity and found to produce 2.8×10^6 units of activity per ml when assayed on BT2 cells. This corresponds to 24 μg of recombinant IL-2 per ml of *E. coli* culture estimated on the basis of the specific activity (1.2×10^5 units/μg) of purified recombinant bovine IL-2 produced in yeast (D. L. Urdal, V. Price, R. Klinke, C. J. March, S.G., and P.E.B., unpublished results). Control plasmids, lacking IL-2 sequences (Fig. 3, lane 1), yielded no appreciable biological activity (< 0.1 unit/ml).

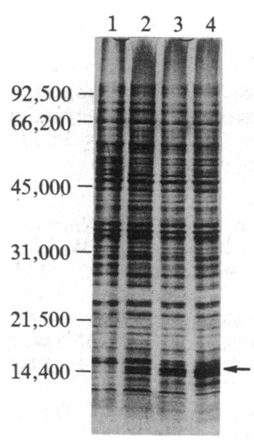

FIG. 3. Induction of bovine IL-2 synthesis in *E. coli*. Aliquots of *E. coli* cultures were centrifuged and prepared for $NaDodSO_4$/PAGE at the times after induction listed below. Lanes: 1, RR1(pRK248cIts) with a control plasmid containing P_L lacking bovine IL-2 sequences, 20 hr; 2, RR1(pRK248cIts) (pLNBovIL2), 1.5 hr; 3, RR1-(pRK248cIts) (pLNBovIL2), 4 hr; 4, RR1(pRK248cIts) (pLNBovIL2), 20 hr. The positions of protein molecular weight markers are indicated at the far left. Arrow indicates position of recombinant bovine IL-2.

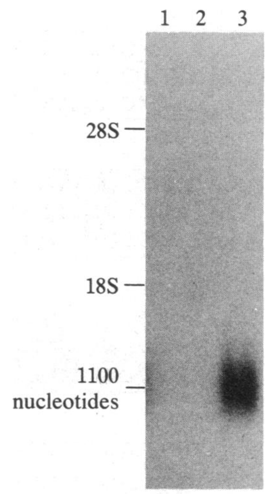

FIG. 4. Autoradiograph of blots of RNA from lymph node cells hybridized with IL-2 probe. Lanes: 1, 4 μg of polyadenylylated RNA from unstimulated cells; 2, 4 μg of polyadenylylated RNA from unstimulated cells cultured for 17 hr; 3, 4 μg of polyadenylylated RNA from stimulated cells cultured for 17 hr with Con A. The positions of 18S and 28S rRNA bands are indicated.

Analysis of mRNA. The expression of IL-2 mRNA was studied by blot hybridization analysis of mRNA isolated from Con A-stimulated and unstimulated bovine lymph node cells, using a bovine IL-2 probe (Fig. 4). A single RNA species of approximately 1100 nucleotides was detected in lymph node cells stimulated with Con A but not in unstimulated lymph node cells. The size of bovine IL-2 mRNA is very similar to the size of human and murine IL-2 mRNA (9–14).

Analysis of Genomic Sequences. To determine the number of IL-2-related genes in bovine DNA, a ^{32}P-labeled bovine IL-2 probe was hybridized to Southern blots of genomic DNA digested with three different restriction endonucleases. Digestion with *Bam*HI, *Eco*RI, or *Hin*dIII resulted in one (10.0 kbp), two (5.8, 5.0 kbp), or three (5.1, 3.3, 1.9 kbp) bands, respectively (Fig. 5). These results indicate that the

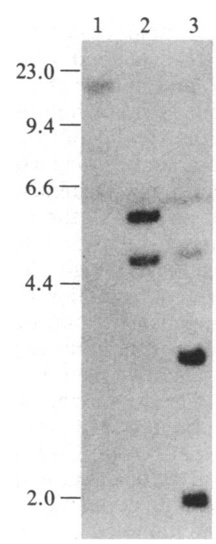

FIG. 5. Autoradiograph of hybridizations with IL-2 cDNA probe to Southern blots of bovine genomic DNA. Genomic DNA (10 μg) was digested with *Bam*HI (lane 1), *Eco*RI (lane 2), or *Hin*dIII (lane 3), electrophoresed in a 0.7% agarose gel, blotted, and hybridized at high stringency to nick-translated ^{32}P-labeled IL-2 cDNA. The molecular size markers (in kbp) are from *Hin*dIII-digested bacteriophage λ DNA.

```
                                                                          36
human    MYRMQLLSCI ALSLALVTNS APTSSSTKKT QLQ....... .......LEH
bovine   MYKIQLLSCI ALTLALVANG APTSSSTGNT MKE....... .......VKS
murine   MYSMQLASCV TLTLVLLVNS APTSSSTSSS TAEAQQQQQQ QQQQQQHLEQ

                                                                          86
         LLLDLQMILN GINNYKNPKL TRMLTFKFYM PKK.ATELKH LQCLEEELKP
         LLLDLQLLLE KVKNPENLKL SRMHTFDFYV PKVNATELKH LKCLLEELKL
         LLMDLQELLS RMENYRNLKL PRMLTFKFYL PKQ.ATELKD LQCLEDELGP

                                                                         136
         LEEVLNLAQS KNFHL.RPRD LISNINVIVL ELKGSETTFM CEYADETATI
         LEEVLNLAPS KNLNPREIKD SMDNIKRIVL ELQGSETRFT CEYDDATVNA
         LRHVLDLTQS KSFQLEDAEN FISNIRVTVV KLKGSDNTFE CQFDDESATV

                                                                         155
         VEFLNRWITF CQSIISTLT
         VEFLNKWITF CQSIYSTMT
         VDFLRRWIAF CQSIISTSPQ
```

FIG. 6. Alignment of human (9), bovine, and murine (13) IL-2 amino acid sequences as deduced from their DNA sequences. Numbering, starting at the initiator Met, is for bovine IL-2. Boxed residues indicate homology at two or more residues. The star represents the predicted amino terminus of mature IL-2. Arrows identify the conserved Cys residues, with the heavy arrows indicating those involved in formation of an active conformation, and the triangle indicates the possible N-linked glycosylation site in bovine IL-2.

gene for bovine IL-2 probably exists as a single copy, as was predicted for human IL-2 (9, 12, 31).

DISCUSSION

Using a human IL-2 cDNA probe, we isolated the homologous bovine gene from a cDNA library. To prove that this cDNA encoded bovine IL-2, we constructed an expression plasmid designed to direct the synthesis of this protein in *E. coli* and demonstrated biological activity with a bovine IL-2-responsive cell line.

Analysis of the amino acid sequence, as deduced from the nucleotide sequence, reveals that mature bovine IL-2 is composed of 135 amino acids and has a molecular weight of 15,450. This may be compared with the estimated molecular weight of 14,400–25,000 of IL-2 isolated from lymph node cells. The heterogeneity in molecular weight has been attributed to variable degrees of glycosylation (8). The amino acid sequence of bovine IL-2 aligned with the amino acid sequences of human (9) and murine (13) IL-2 is shown in Fig. 6. The bovine protein is more homologous to the human (approximately 65%) than to the murine (approximately 50%) sequence and this homology is dispersed throughout. However, one region, the amino terminus of mature IL-2, has seven identical amino acids in all three proteins. In addition, the positions of all three Cys residues are conserved, including the two Cys residues thought to be involved in the formation of an active conformation (13). Bovine IL-2, like the human homolog, lacks the unusual stretch of 12 Gln residues that is present in the murine polypeptide. Thus, it was not surprising that a human IL-2 cDNA probe was capable of hybridizing to the bovine homolog and therefore useful in isolating the bovine IL-2 cDNA clone. However, it is of interest to note that bovine IL-2 is unique in that it has one potential N-linked glycosylation site. This is due to an insertion of an Asn residue at position 70 that is absent from the human and murine homologs.

In the past there has been some controversy about the spectrum of activities that bovine IL-2 displayed in different species. For example, it has been reported that bovine IL-2 would (32) or would not (33) induce murine IL-2-responsive T-cell lines to proliferate. The availability of recombinant bovine IL-2 has allowed us to reexamine this question. We have found that the murine IL-2-responsive T-cell line CTLL-2 is totally refractory to stimulation with recombinant bovine IL-2. On the other hand, since bovine IL-2 used in these experiments was produced in *E. coli*, it remains possible that the potential N-linked glycosylation site (i.e., Asn-70) could in some way alter its effect on murine T-cell proliferation. However, it should be stressed that recombinant bovine IL-2, as produced in a yeast expression vector capable of N-linked glycosylation (D. L. Urdal, V. Price, R. Klinke, C. J. March, S.G., and P.E.B., unpublished results), is also unable to promote murine T-cell proliferation.

Human recombinant IL-2 will induce proliferation of murine, as well as bovine, T-cell lines (23, 34). This has led to speculation that recombinant human IL-2 might find useful application in cattle. However, in light of the significant disparity between the human and bovine amino acid sequences, it is likely that human IL-2 would be antigenic in cattle. Thus, the availability of sizable quantities of recombinant bovine IL-2 should make it possible to ascertain potential therapeutic or prophylactic utility of this lymphokine in cattle.

We thank Toni Read for preparation of the manuscript, Bruce Acres and Ken Grabstein for RNA preparations, and Steve Gimpel, Janis Wignall, Cathy Grubin, Kathy Picha, and June Little for excellent technical support.

1. Gillis, S. & Smith, K. A. (1977) *Nature (London)* **268,** 154–156.
2. Mittler R., Rao, P., Olini, G., Westberg, E., Newman, W., Hoffmann, M. & Goldstein, G. (1985) *J. Immunol.* **134,** 2393–2399.
3. Boyd, A. W., Fisher, D. C., Fox, D. A., Schlossman, S. F. & Nadler, L. M. (1985) *J. Immunol.* **134,** 2387–2392.
4. Ekstedt, R. D. & Meridan, D. J. (1984) *Cell. Immunol.* **85,** 447–458.
5. Cheever, M. A., Greenberg, P. D. & Fefer, A. (1981) *J. Immunol.* **126,** 1318–1322.
6. Mills, G. B., Carlson, G. & Paetkau, V. (1980) *J. Immunol.* **125,** 1904–1909.
7. Spiess, P. J. & Schwarz, S. (1983) *Transplantation* **35,** 631–634.
8. Robb, R. J. (1984) *Immunol. Today* **5,** 203–209.
9. Taniguchi, T., Mitsui, H., Fujita, T., Tukaoka, C., Kashima, N., Yoshimoto, R. & Hamuro, J. (1983) *Nature (London)* **302,** 305–310.
10. Devos, R., Plaetinck, G., Cherotre, H., Simons, G., Degrave,

W., Tavernier, J., Remaut, E. & Fiers, W. (1983) *Nucleic Acids Res.* **11,** 4307–4323.
11. Maeda, S., Nishino, N., Obaru, K., Mita, S., Nomiyama, H., Shimada, K., Fujimoto, K., Teranishi, T., Hirano, T. & Onoue, K. (1983) *Biochem. Biophys. Res. Commun.* **115,** 1040–1047.
12. Clark, S. C., Arya, S. K., Wong-Staal, F., Matsumoto-Kobayashi, M., Kay, R. M., Kaufmann, R. J., Brown, E. L., Shoemaker, C., Copeland, T., Oroszlan, S., Smith, K., Sarngadharan, M. G., Linder, S. G. & Gallo, R. C. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2543–2547.
13. Kushima, N., Nishi-Takaoka, C., Fujita, T., Taki, S., Yamada, G., Hamuro, J. & Taniguchi, T. (1985) *Nature (London)* **313,** 402–404.
14. Yokota, T., Arai, N., Lee, F., Rennick, D., Mosmann, T. & Arai, K.-I. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 68–72.
15. Namen, A. E. & Magnuson, J. A. (1984) *Immunology* **52,** 469–475.
16. Cosman, D., Cerretti, D. P., Larsen, A., Park, L., March, C., Dower, S., Gillis, S. & Urdal, D. (1984) *Nature (London)* **312,** 768–771.
17. March, C. J., Mosley, B., Larsen, A., Cerretti, D. P., Braedt, G., Price, V., Gillis, S., Henney, C. S., Kronheim, S. R., Grabstein, K., Conlon, P. J., Hopp, T. P. & Cosman, D. (1985) *Nature (London)* **315,** 641–647.
18. Norrander, J., Kempe, T. & Messing, J. (1983) *Gene* **26,** 101–106.
19. Sanger, F., Nicklen, B. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
20. Biggin, M. D., Gibson, T. J. & Hong, G. F. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 3963–3965.
21. Marquis, D., Smolec, J. M. & Katz, D. H. (1985) *J. Cell. Biochem.* Suppl. 9B, abstr. 990, 221.
22. Jay, G., Khoury, G., Seth, A. K. & Jay, E. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5543–5548.
23. Laemmli, U. K. (1970) *Nature (London)* **227,** 680–685.
24. Oakley, B. R., Kirsch, D. R. & Morris, N. R. (1980) *Anal. Biochem.* **105,** 361–363.
25. Picha, K. & Baker, P. (1986) *Immunology* **57,** 131–136.
26. Gillis, S., Ferm, M., Ou, W. & Smith, K. (1978) *J. Immunol.* **120,** 2027–2032.
27. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) in *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
28. Wickens, M. & Stephenson, P. (1984) *Science* **226,** 1045–1051.
29. Robb, R. J., Kutny, R. M., Panico, J., Morris, H. R. & Chowdkry, V. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6486–6490.
30. Watson, M. E. E. (1984) *Nucleic Acids Res.* **12,** 5145–5164.
31. Holbrook, N. J., Smith, K. A., Fornace, A. J., Jr., Comeau, C. M., Wiskocil, R. L. & Crabtree, G. R. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1634–1638.
32. Miller-Edge, M. & Splitter, G. A. (1984) *Vet. Immunol. Immunopathol.* **7,** 119–130.
33. Baker, P. & Knoblock, K. (1982) *Vet. Immunol. Immunopathol.* **3,** 381–397.
34. Brown, W. C. & Grab, D. J. (1985) *J. Immunol.* **135,** 3184–3190.